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A novel fluorescent probe for the detection of AmpC beta-lactamase and the application in screening beta-lactamase inhibitors

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ABSTRACT

The rapid detection of β-lactamases (Blas) and effective screening of Bla inhibitors are critically important and urgent for solving antibiotic resistance and improving precision medicine. Here a novel fluorescent probe **CDC-559** was designed and synthesized, which can be used for the selective and direct detection of AmpC Blas. More importantly, it can realize screening the Bla inhibitors with sulbactam sodium and tazobactam as model compounds, and the half-maximal inhibitory concentration are 0.279 μM and 0.053 μM, respectively. **CDC-559** can be applied not only to examine the resistance of bacterial strains, but also to categorize its mode of action specifically, which is consistent with the essential result of the Blas. The research suggests that **CDC-559** probe has tremendous potential in the rapid detection of AmpC Blas as well as the strains with AmpC-encoded gene, which is instructive in promoting better antibiotic stewardship practices and developments.

1. Introduction

β-Lactam antibiotics are the most widely used medications in clinic globally due to the advantages of strong bactericidal activity by inhibition of cell wall synthesis, low toxicity, wide indications, and good clinical efficacy. Therefore, β-lactams have been known for their excellent therapeutic efficacy and broad-spectrum applicability to bacterial infection diseases since their first use in the 1940s [1–3]. With the overuse and misuse of β-lactam antibiotics, resistance to β-lactams that occurs via acquisition of plasmids that encode various mechanisms of resistance, such as reduced access to Penicillin-binding proteins (PBPs), mutation of PBPs, and overexpression of β-lactamase (Blas), etc. [4–11] have become more and more serious. The phenomenon of antibiotic resistance in bacterial pathogens is a persistent threat and a global public concern in society [12]. Among the mechanisms of β-lactam resistance, the most significant mechanism in both gram-negative and gram-positive bacteria is the expression of β-lactamases, which can hydrolyze most of β-lactam antibiotics to deactivated forms. These Blas can be categorized into four different classes (A, B, C, and D) based on the Ambler classification [13–16]. Class A Blas, such as TEM-1 Blas, have been well studied, including its interaction with antibiotics and detection methods [17–23]. On the contrary, AmpC Blas that can primarily hydrolyze the third generation cephalosporins and can also hydrolyze ampicillin and cefoxitin [24] are less well understood. Compared with TEM-1 Blas, the binding pocket in AmpC Blas is looser, which can allow substrates with greater steric hindrance to enter, and thus results in a wider resistance effect on antibiotics. AmpC Blas are induced easily by the long-term use of cephalosporins, which makes the bacteria less susceptible to β-lactam antibiotics and brings a new challenge to clinical anti-infection treatments. Accurate detection and categorization of drug-resistant bacteria are useful for timely and correct selection of antibiotics clinically. Therefore, the rapid detection of AmpC Blas should be put on the agenda. Some fluorescent probes have been developed for detecting the Blas [18–23,25–28], but the studies on the detection methods of AmpC Blas are relatively rare [29,30], and have not been used in clinical examinations.

To combat antibiotic resistance induced by Blas, some related Bla inhibitors, such as clavulanic acid, sulbactam, tazobactam, and avibactam, have been developed as they can dramatically inhibit Bla activities

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1386-1425/

and thus conserve the validity of β-lactam antibiotics [31–34]. As a result, the combination of Bla inhibitors and β-lactam antibiotics against bacterial resistance caused by Blas has become one of the common strategies for treating infection in clinic, which has been proven to be very effective by numerous clinical trials and applications. In this paper, a fluorogenic probe **CDC-559** has been designed and applied to examine AmpC Bla specifically, and more importantly, it can be used to rapidly screen AmpC Bla inhibitors.

2. Experimental

2.1. Materials and instruments

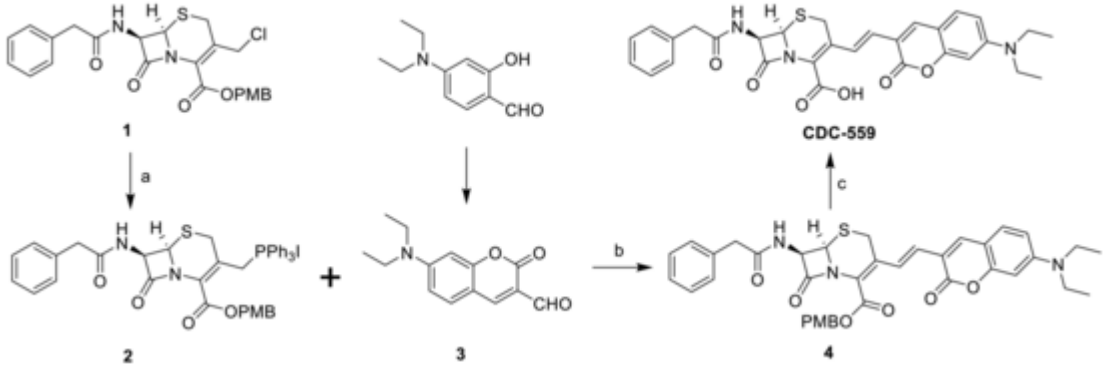
4-Methoxybenzyl-3-chloromethyl-7-(2-phenylacetamido)-3-cephem-4-carboxylate (**1**) was purchased from Shanghai BidePharmatech Company Ltd., and 4-(diethylamino)salicylaldehyde was purchased from Energy Chemical Company Ltd. (Shanghai). All other chemicals were obtained commercially and used without further purification unless otherwise noted. AmpC Bla was purchased from Shanghai Aladdin Biochemical Technology Company Ltd. and TEM-1 Bla was acquired from Shanghai Yuanye Biotechnology Company Ltd. All strains were purchased from Guangdong Culture Collection Center. Column-layer chromatographic silica gel was purchased from Branch of Qingdao Haiyang Chemical Co., Ltd. HEPES buffer (pH 7.4) consisted of 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 100 mM NaCl, 0.01% polyethylene glycol *tert*-octylphenyl ether (Triton X-100) and 1 $\mu g \cdot mL^{-1}$ benzenesulfonamide. ^1H and ^{13}C NMR spectra were recorded using Bruker AVANCE II 400 M/600 M (Bruker, Germany) spectrometer. High resolution mass spectra (HRMS) were measured with maXis impact (Bruker, Germany). Fluorescence spectra and kinetic experiments were performed on a multimode microplate reader (EnSpire-2300, US) or a F-4500 fluorescence spectrophotometer (Hitachi, Japan). UV absorption spectra were recorded on a UV–Vis 2450 spectrophotometer (SHIMADZU, Japan).

2.2. Synthesis of CDC-559

The synthetic route of probe CDC-559 was shown in Scheme 1. 7-Diethylamino-3-formylcoumarin **3** was synthesized from 4-(diethylamino) salicylaldehyde in 60% yield, and the result was in agreement with that published previously [35]. ESI-HRMS (*m/z*) Calcd. for $C_{14}H_{15}NO_3$ 245.1052, found: 268.0948 $[M + Na]^+$ (Calcd. 268.0950). ^1H NMR (400 MHz, $CDCl_3$) δ 10.13 (s, 1H), 8.26 (s, 1H), 7.41 (d, J = 9.0 Hz, 1H), 6.64 (dd, J = 9.0, 2.4 Hz, 1H), 6.49 (d, J = 2.3 Hz, 1H), 3.48 (q, J = 7.1 Hz, 4H), 1.26 (t, J = 7.1 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 187.94, 161.91, 158.94, 153.51, 145.42, 132.54, 114.25, 110.23, 108.23, 97.14, 45.29, 12.46.

Ylide salt **2** was prepared according to the literature reported previously [36]. 4-Methoxybenzyl-3- chloromethyl-7-(2-phenylacetamido)-3-cephem-4-carboxylate (GCLE, **1**) (3.0 g, 6.1 mmol) and NaI (4.0 g, 26.7 mmol) were stirred in acetone (40 mL) at room temperature for 1 h. After removal of acetone, 100 mL ethyl acetate was added and the organic layer was washed with 10% $Na_2S_2O_3$ (50 mL × 1), brine (50 mL × 1), water (50 mL × 1), and dried over with anhydrous $MgSO_4$. After the solid was removed, the resulting solution was then concentrated under reduced pressure to about 40 mL. Triphenylphosphine (1.9 g, 7.2 mmol) was added rapidly at room temperature under a nitrogen atmosphere, and light-yellow solid was formed after 12 h. After filtered and washed with hexane (10 mL × 3), the solid was dried to afford ylide salt **2** (4.0 g, 77%) for the further usage. Ylide salt **2** (4.0 g) was dissolved in dichloromethane (70 mL) and stirred vigorously with 1 M NaOH solution (43 mL) for 30 min at ambient temperature. The organic phase was separated and dried with anhydrous $MgSO_4$. After filtration, the resulting filtrate was combined with 7-diethylamino-3-formylcoumarin (**3**) (0.8 g in 20 mL dichloromethane). The mixture was maintained stirring at room temperature for 6 h. After evaporation, the crude product was purified by flash chromatography on silica gel (eluted: dichloromethane) to afford **4** as a red solid (0.62 g, 28%). HRMS (ESI, *m/z*) Calcd. for $C_{38}H_{37}N_3O_7S$ 679.2352, found: 702.2254 $[M + Na]^+$ (Calcd. 702.2250). ^1H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, J = 16.5 Hz, 1H), 7.58 (s, 1H), 7.40–7.21 (m, 8H), 6.91–6.83 (m, 3H), 6.57 (d, J = 8.6 Hz, 1H), 6.43–6.38 (m, 2H), 5.81 (dd, J = 9.1, 4.8 Hz, 1H), 5.25 (q, J = 12.1 Hz, 2H), 4.96 (d, J = 4.7 Hz, 1H), 3.78 (s, 3H), 3.66 (m, 2H), 3.65 (d, J = 2.8 Hz, 2H), 3.42 (q, J = 6.9 Hz, 4H), 1.22 (t, J = 6.9 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.20, 164.67, 162.05, 161.56, 159.85, 155.96, 150.89, 137.77, 133.86, 130.69, 129.45, 129.38, 129.13, 128.57, 127.66, 127.08, 126.61, 123.70, 123.18, 116.69, 114.01, 109.29, 108.92, 97.12, 67.87, 59.17, 57.90, 55.26, 44.92, 43.35, 24.52, 12.53.

Anisole (200 μL) and trifluoroacetic acid (1 mL) in dichloromethane (5 mL) were mixed and cooled to 0 °C. Compound **4** (100 mg, 0.15 mmol) was added, and kept stirring for 1 h at 0 °C. After removal of the solvent under reduced pressure, cold ether (2 mL) was added to produce orange precipitate, filtered and the residue was washed with ether (1 mL × 3) to afford probe **CDC-559** as red solid (24 mg, 30%). HRMS (ESI, *m/z*) calcd. for $C_{30}H_{29}N_3O_6S$ 559.1777, found 582.1669 $[M + Na]^+$ (Calcd. 582.1675). ^1H NMR (600 MHz, DMSO-d_6) δ 9.09 (d, J = 8.2 Hz, 1H), 7.76 (s, 1H), 7.68 (d, J = 16.6 Hz, 1H), 7.47 (d, J = 8.7 Hz, 1H), 7.27 (m, 6H), 6.70 (d, J = 8.3 Hz, 1H), 6.53 (s, 1H), 6.43 (d, J = 16.6 Hz, 1H), 5.48 (dd, J = 7.6, 4.8 Hz, 1H), 5.00 (d, J = 4.3 Hz, 1H), 3.65–3.49 (m, 4H), 3.43 (q, J = 6.9 Hz, 4H), 1.13 (t, J = 6.6 Hz, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.44, 164.85, 163.85, 160.36, 155.84, 151.15, 141.18, 136.31, 130.18, 129.50, 128.70, 126.97, 125.64, 125.47, 125.39, 116.20, 109.92, 108.81, 96.69, 65.39, 59.72, 58.38, 44.64, 42.07, 31.16, 23.93, 15.64, 12.84.



Scheme 1. Reagents and conditions: (a) NaI, triphenylphosphine, rt., 1 h, 77%; (b) NaOH, rt., 6 h, 28%; (c) anisole, CF_3COOH, 0 °C, 1 h, 30%.

2.3. General spectral measurements

The **CDC-559** stock solution (10 mM) was prepared in DMSO. Stock solutions of sulbactam sodium (10 mM) and tazobactam (10 mM) were prepared in deionized water. TEM-1 Bla (1 U = 1.10×10^{-12} mol) and AmpC Bla (1 U = 1.54×10^{-12} mol) were diluted to desired concentration with HEPES buffer (pH 7.4), respectively. All stock solutions were made freshly for typical measurements. For general spectral measurements, probe **CDC-559** was diluted to 10 μM with HEPES buffer (pH 7.4). The UV spectrum and fluorescence property were measured at room temperature as follows. **CDC-559** in HEPES buffer was added to a quartz cuvette with or without AmpC or TEM-1 Blas, then the total volume was adjusted to 3 mL to provide the final concentrations of 10 μM for **CDC-559**, 100 U·mL^{-1} for AmpC Bla and 100 U·mL^{-1} for TEM-1 Bla, respectively. The UV spectrum was measured for a total of 10 min with the time interval of 1 min, and the fluorescence property was recorded on a F-4500 fluorescence spectrophotometer when excited at 440 nm.

2.4. Representative procedure for the determination of enzymatic kinetic parameters

Kinetic assays were performed by using the multimode microplate reader (EnSpire-2300, US) at 37 °C. AmpC Bla (62.5 U·mL^{-1}) or TEM-1 Bla (12,500 U·mL^{-1}) in HEPES buffer was added to the 96-well plate (black and flat bottom) separately, five different concentrations of **CDC-559** (1, 1.25, 2.5, 5 and 10 μM) was added subsequently to start reaction. Reactions were monitored for a total of 1 min, with data points recorded every 10 s, and initial velocity slopes were calculated in 1 min (λ_{ex} = 440 nm, λ_{em} = 539 nm). Steady state kinetic parameters (K_m and k_{cat}) were determined from three independent measurements and five different substrate concentrations by fitting the initial velocity data to the Michaelis-Menten equation using Origin software.

2.5. Evaluation of the inhibition activity of Bla inhibitors by IC_{50}

IC_{50} assays were performed by using the multimode microplate reader (EnSpire-2300, US) at 37 °C. The concentration of AmpC Bla was fixed at 62.5 U·mL^{-1}. To a 96-well plate was added AmpC Bla in HEPES buffer, a series concentrations of sulbactam sodium (2.50, 1.25, 0.625, 0.313, 0.156, 0.0783, 0.0391 μM) or tazobactam (0.50, 0.25, 0.125, 0.0625, 0.0313, 0.0156, 0.00783 μM) was added subsequently, the resulting mixture was incubated at room temperature for 10 min. And **CDC-559** (15 μM) was added to start reaction. Reactions were monitored for a total of 1 min with data points recorded every 6 s, and initial velocity slopes were calculated in 36 s (λ_{ex} = 440 nm, λ_{em} = 539 nm). The experimental value of each point is the average of the three repeated wells. The IC_{50} was obtained by Origin software.

2.6. Representative procedure for limit of detection (LOD)

A series of AmpC Bla concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 U·mL^{-1}) in HEPES buffer (pH 7.4) was added to the 96-well plate (black and flat bottom) separately, and then **CDC-559** (10 μM) was added to start reaction. The fluorescence intensity was recorded (λ_{ex} = 440 nm, λ_{em} = 539 nm) on the multimode microplate reader after the resulting mixture was incubated at 37 °C for 30 min. The experimental value of each point is the average of the three repeated wells.

2.7. Specificity study and categorization evaluation in standard strains

Two susceptible strains (*Staphylococcus aureus* ATCC 25923 and ATCC 29213) and two resistant bacteria (*Staphylococcus aureus* ATCC 43300 and *Enterobacter cloacae* ATCC 13047) were selected to investigate the specificity and categorization effect of **CDC-559**. ATCC 43300 was resistant mainly due to carrying an encoded gene to express PBP2a protein, and ATCC 13047 was resistant due to the native capability to produce AmpC Bla in large quantities. These bacterial cultures were re-grown into fresh medium overnight until OD_{600} reached around 0.5. The cultures were further diluted 10 times, and then spread over the entire solid medium. Five discs with 1 mg different ingredients (Oxacillin sodium (1), **CDC-559** (2), GCLE (3), cefazolin sodium (4) and the solvent (99% deionized water and 1% DMSO, 5)) were placed on the medium and incubated for 16 h at 37 °C. In addition, the bacterial cultures with an OD_{600} value of about 0.5 were ultrasonicated, supernatant (15 μL) was added to the **CDC-559** solution (10 μM) to test the bacterial resistance against AmpC, respectively.

3. Result and discussion

3.1. Design of the probe

In a general chemical structure of Bla fluorescence probes reported previously [37–39], the β-lactam pharmacophore and fluorophore were always connected by a C-N/C-O/C-S single-bond, which could be cleaved by Blas to release fluorophore resulting in fluorescence response. Herein, we designed a novel "C=C double bond-linked" fluorescence probe **CDC-559** in which the 7-diethylaminocoumarin and the 3-cephem-4-carboxylate was chemically connected by a carbon-carbon double bond, which was different from the previous probes in principle. In our designed probe, the addition of AmpC Blas provided a deactivated ring-opened molecule through the hydrolysis of the β-lactam ring, and the β-lactam nitrogen atom was changed from a weak electron donor to a strong amine donor, but not the elimination of the side chain and formation of a conjugated imine. This phenomenon could bring about a large decrease in fluorescence intensity after hydrolysis (Fig. 1a). To prove our assumption, the enzymatic hydrolysate, **CDC-559-2**, was characterized by ESI-HRMS (Fig. 1b). The mass spectrum showed that the negative fragment at 576.1814 (*m/z*) corresponds to [**CDC-559** + H_2O-H]$^-$ (calcd. For $C_{30}H_{30}N_3O_7S$: 576.1804), which was also accordance with the theoretical isotopic distribution pattern (ESI, Fig. S1).

3.2. Optical responses of CDC-559 towards β-lactamase

The spectral properties and the response of **CDC-559** to Blas were firstly investigated. The UV–Vis spectrum of **CDC-559** (10 μM) showed a maximal absorption peak at 440 nm with yellow color in HEPES buffer (Fig. 2a). The maximal absorption wavelength did not present obvious change with the addition of AmpC Bla, but the absorption intensity decreased gradually in time. The fluorescence spectrum of **CDC-559** featured a strong emission peak at 539 nm (excited at 440 nm), and the intensity decreased significantly (up to about 93%) after the incubation with AmpC Bla (at 100 U mL^{-1}, Fig. 2b) in 10 min. Significant change in fluorescence intensity was particularly meaningful for the enzyme detection and inhibitor screening. However, when adding the equal amount of TEM-1 Bla (100 U mL^{-1}) to the same **CDC-559** solution (10 μM), both maximal absorption wavelength and fluorescence intensity almost did not change at all (Fig. 2c and d) in 10 min. These data clearly demonstrated that **CDC-559** showed higher selectivity to AmpC Blas than to TEM-1 Blas, which might be attributed to the short linker at the 3′-position of the cephalosporin structure that increased steric hindrance in blocking the active site of TEM-1 Blas, and that **CDC-559** could fit better with AmpC Blas through its larger binding pocket.

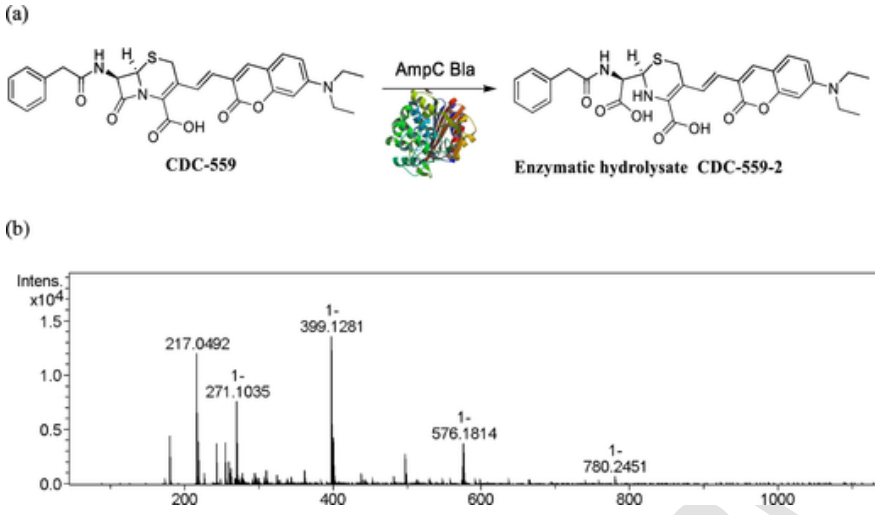


Fig. 1. (a) The reaction scheme of **CDC-559** with AmpC Bla; (b) ESI-HRMS of **CDC-559** (100 μg mL^{-1}) hydrolysis with AmpC Bla (1000 U mL^{-1}).

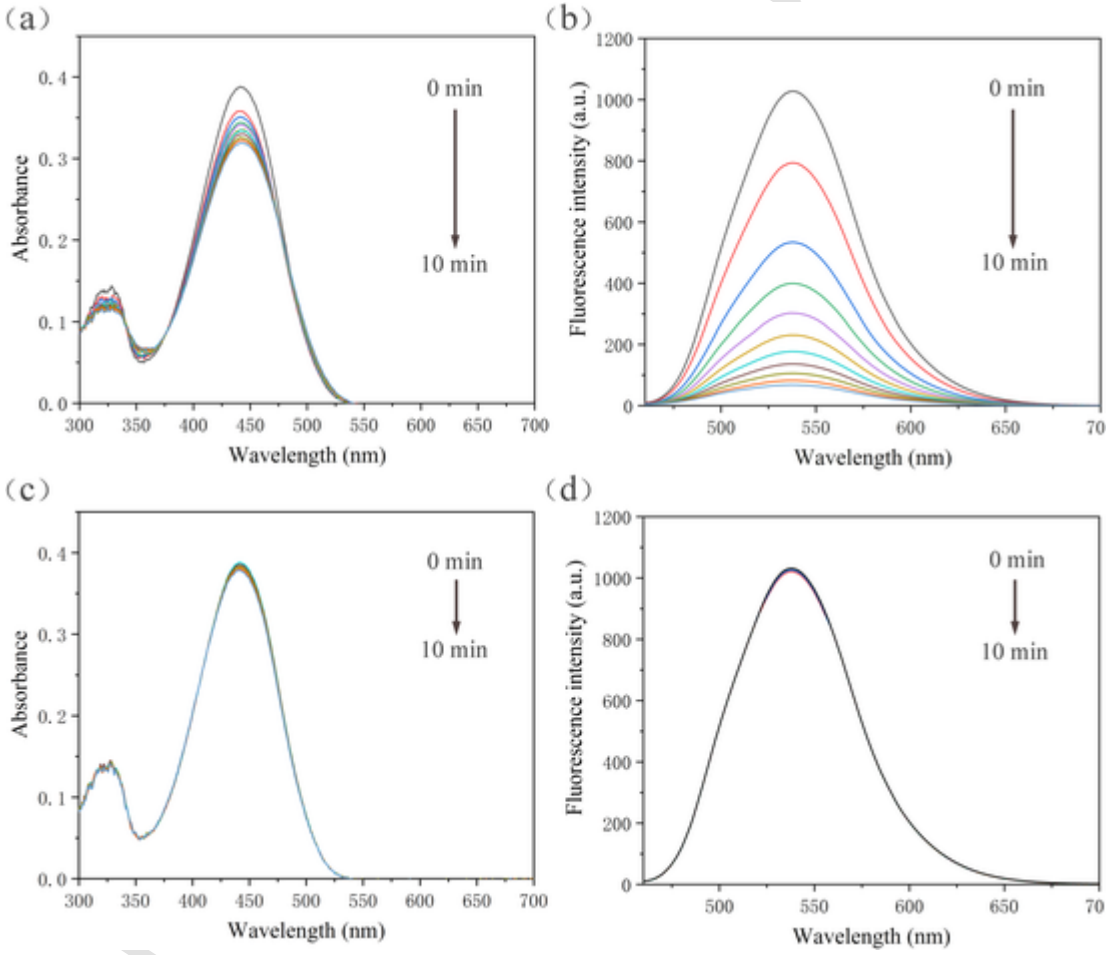


Fig. 2. The change of UV–vis absorption and fluorescence spectra of **CDC-559** (10 μM) upon the addition of Blas (100 U mL^{-1}). (a) UV–vis and (b) fluorescence spectra for AmpC Bla; (c) UV–vis absorption and (d) fluorescence spectra for TEM-1 Bla.

3.3. Enzymatic kinetic parameters

When **CDC-559** as a substrate of Blas, kinetic parameters for both AmpC and TEM-1 Blas were determined by Lineweaver-Burk plots (Fig. 3). The slope of the straight-line equation in Lineweaver-Burk plot for AmpC Bla was 243.41 ± 3.43, and the intercept on the Y axis was 5.16 ± 0.71, which resulted in the K_m value of 47.2 ± 0.7 μM and v_{max} was calculated as 0.194 ± 0.024 μM s^{-1}, so $k_{cat} = v_{max}/[E] = 2.0 \pm 0.2\ s^{-1}$ (62.5 U mL^{-1} = 96.3 nM). Catalytic efficiency k_{cat}/K_m was calculated to be $4.2 \times 10^4\ M^{-1}{\cdot}s^{-1}$. When the added enzyme was TEM-1 Bla, the slope of the straight-line equation in Lineweaver-Burk plot was 155.73 ± 7.61, and the intercept on the Y

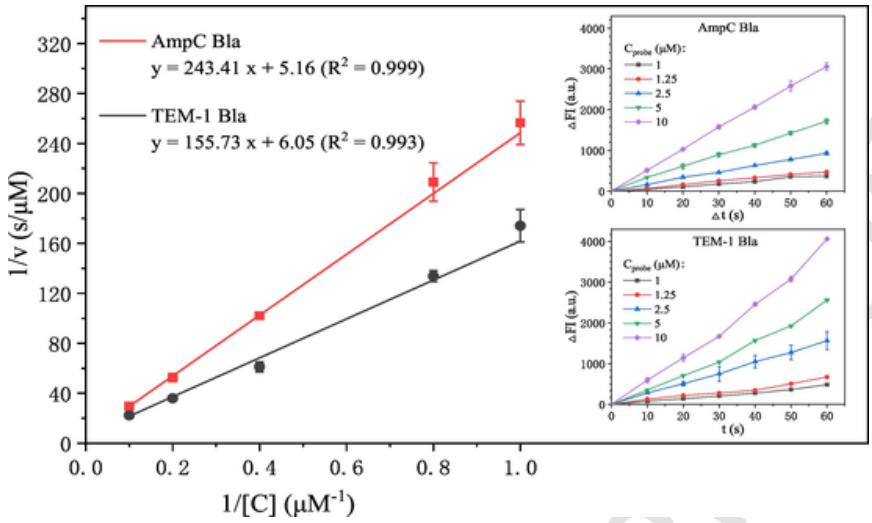


Fig. 3. Lineweaver-Burke plot of AmpC and TEM-1 Blas with CDC-559 as substrate (insert: changes in fluorescence intensity over time at the beginning of the reaction).

axis was 6.05 ± 1.77. So the K_m, k_{cat} and k_{cat}/K_m were found to be 25.7 ± 1.3 μM, 0.012 ± 0.003 s^{-1} and 4.7 × 10^2 $M^{-1}{\cdot}s^{-1}$ according to the same calculation method as AmpC Bla. These results demonstrated that the catalytic efficiency of AmpC Bla towards **CDC-559** was noticeably higher than of TEM-1 Bla, suggesting a greater specificity and catalytic activity of AmpC Bla towards **CDC-559**.

3.4. Limit of detection

Sensitivity is an important parameter for a probe in biological tests. As shown in Fig. 4, with the titration of the concentrations of AmpC Blas to **CDC-559**, the fluorescence intensity gradually decreased and had an excellent linear relationship ($R^2 \geq 0.99$) with AmpC Blas concentration from 0 to 3.5 U mL^{-1}. The limit of detection was determined as 0.377 U mL^{-1} within 30 mins based on 3σ/κ method, indicating that extremely low AmpC Bla concentration could be detected by **CDC-559** probe.

3.5. Screening of Bla inhibitors

Sulbactam sodium and tazobactam were selected to validate our screening assay for AmpC Bla inhibitors. With **CDC-559** as the substrate of AmpC Bla, the initial velocity (v_0) was obtained by the change of the fluorescence intensity in time and the IC_{50} values of sulbactam sodium and tazobactam against AmpC Blas were calculated as 0.279 μM and 0.053 μM, respectively (Fig. 5), proportionally similar to previous reports (11.90 μM and 1.76 μM) [40], showing that **CDC-559** had tremendous potential and sensitivity in screening the Bla inhibitors similar to nitrocefin.

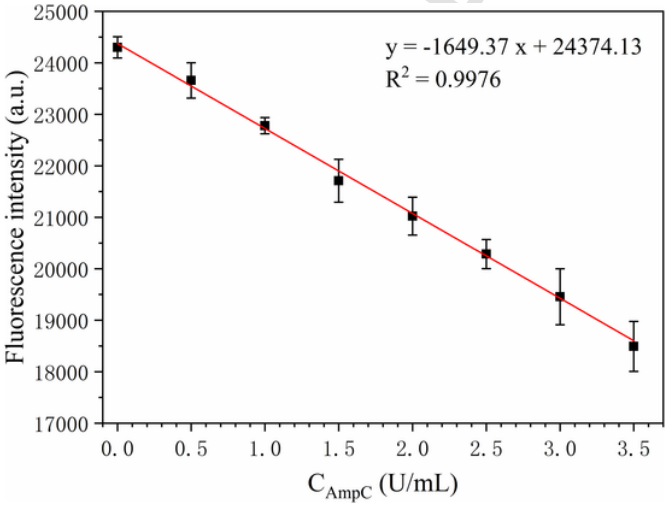


Fig. 4. Linear relationship of fluorescence intensity of **CDC-559** (10 μM) after incubation at room temperature for 30 mins with AmpC Bla (0–3.5 U mL^{-1}).

3.6. Phenotypic resistance categorization/evaluation

Susceptible strains (*Staphylococcus aureus ATCC 25923* and *ATCC 29213*), two resistant bacteria strains (*Staphylococcus aureus ATCC 43300* and *Enterobacter cloacae ATCC 13047*), oxacillin sodium (1), **CDC-559** (2), GCLE (3), cefazolin sodium (4) and the solvent (99% deionized water and 1% DMSO as negative control, 5) were used to test the potential to the susceptibility of different bacterial strains. As shown in Fig. 6, *S. aureus* ATCC 25923 and ATCC 29213 strains were susceptible to oxacillin sodium and cefazolin sodium, and *S. aureus* ATCC 43300 and *E. cloacae* ATCC 13047 were resistant to oxacillin sodium and cefazolin sodium, which validated the assay. The raw material GCLE showed a very small inhibition zone to *S. aureus* ATCC 25923, but no inhibition zone on *S. aureus* ATCC 29213 and the two resistant strains. In contrast, **CDC-559** modified from GCLE showed stronger antibacterial activities for both susceptible strains with only a small reduction in the inhibition zone compared with cefazolin sodium, which is a first generation cephalosporin and had significant antibacterial effect on various bacteria. As we expected, **CDC-559** did not show inhibition zone on cephalosporin-resistant *S. aureus* ATCC 43300 and *E. cloacae* ATCC 13047. The results demonstrated that **CDC-559** could be applied to evaluate the susceptibility of the strains.

In order to further distinguish the resistance mechanism of tested bacteria, the fluorescence response of **CDC-559** to the bacteria growing in liquid culture were investigated, and the spectra showed noticeable differences as demonstrated in Fig. 7. When tested against *S. aureus* ATCC 29213 and ATCC 25923 they had little effect on the fluorescence intensity of **CDC-559**. Against *S. aureus* ATCC 43300 it showed a slight decrease in fluorescence intensity of **CDC-559**, but against *E. cloacae* ATCC13047 it showed a rapid decrease (by about 76%) in fluorescence intensity of **CDC-559** within 10 mins, although both *S. aureus* ATCC 43300 and *E. cloacae* ATCC13047 were cephalosporin-resistant strains. The results clearly indicated that **CDC-559** probe could not only distinguish the susceptibility of bacteria to β-lactams, but also specifically identify the AmpC Bla-mediated resistance.

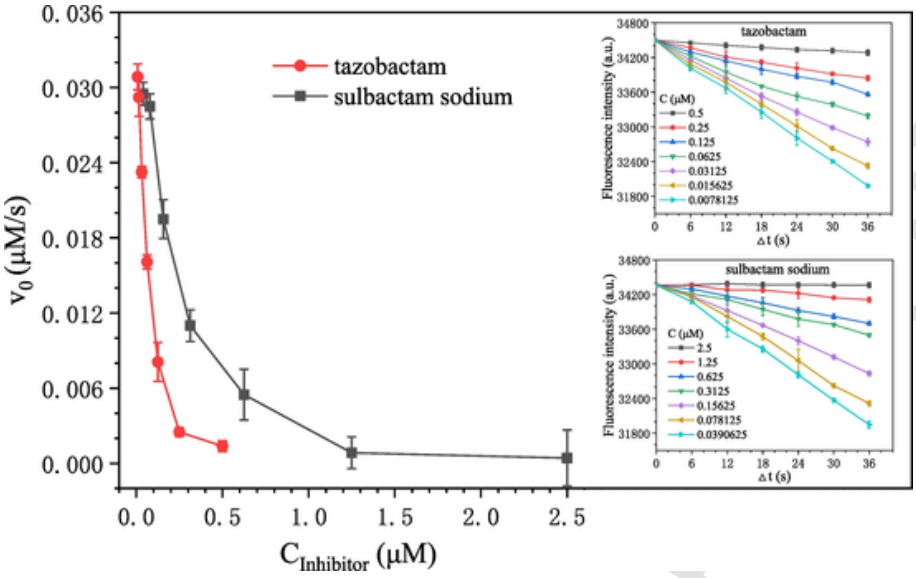


Fig. 5. The determination of IC_{50} of sulbactam sodium and tazobactam against AmpC Blas (insert: changes in fluorescence intensities over time).

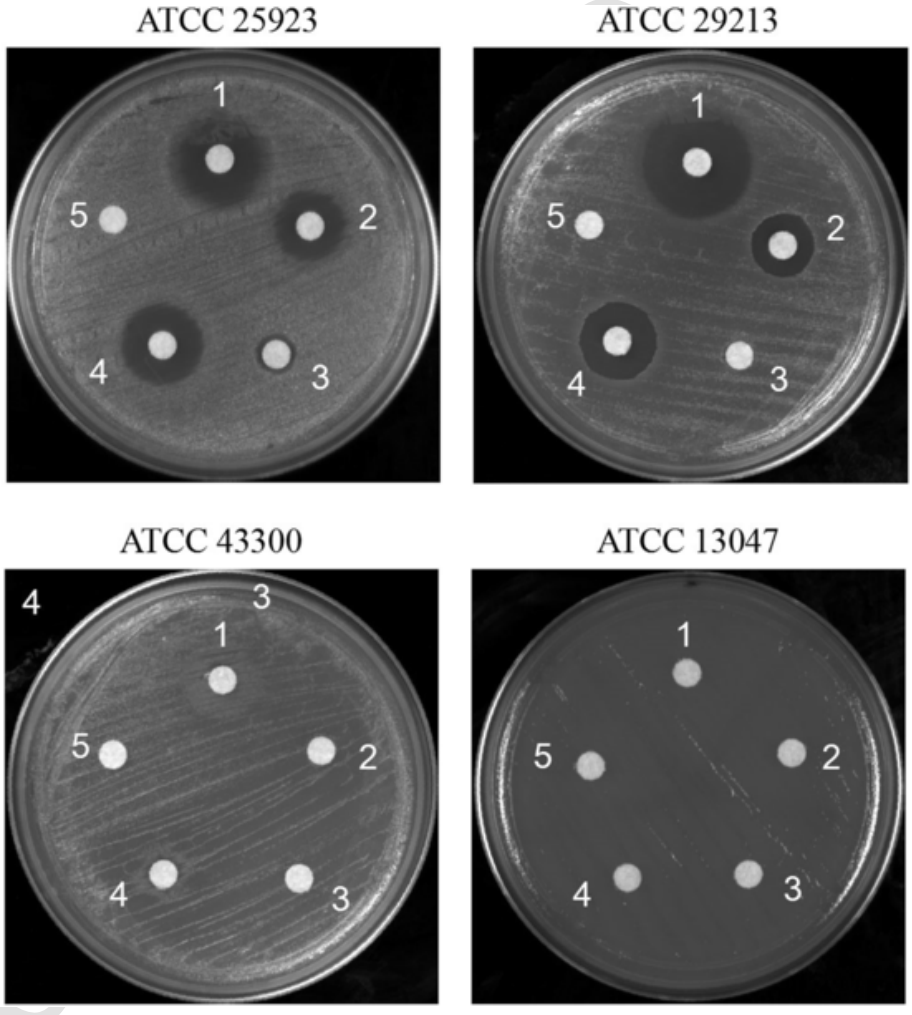


Fig. 6. Pictures of inhibition zone. Every sample is 1 μg on the round paper.

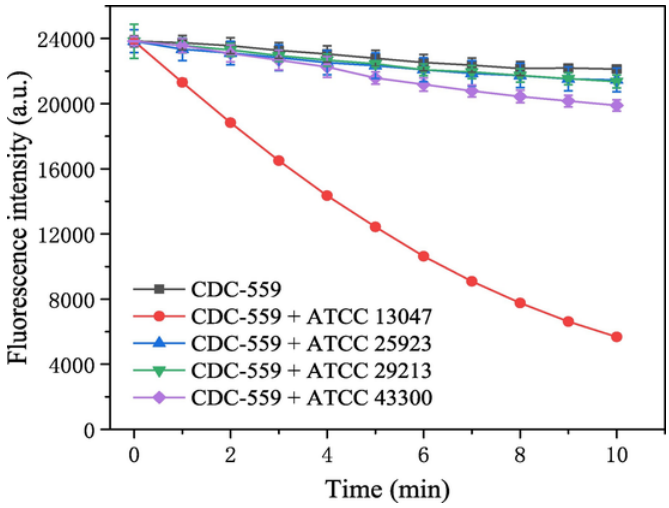


Fig. 7. The change of fluorescence intensity of **CDC-559** (10 μM) upon the addition of the strain solutions (15 μL).

4. Conclusion

In summary, the remarkable increase of antibiotics resistance caused by β-lactamase calls for the rapid susceptibility detection in clinic to aid the selection of antibiotics. In this paper, a novel fluorescence probe **CDC-559** has been designed through linking 7-diethylaminocoumarin and 3-cephem-4-carboxylate with carbon-carbon double bond linker, and synthesized. **CDC-559** probe can specifically recognize AmpC Blas and be applied for selective and efficient detection of AmpC Blas enzymatically and phenotypically. The fluorescence probe showed progressive decrease over time in intensity after being treated with AmpC Blas within 10 mins, and high sensitivity with the LOD of 0.377 U mL^{-1} AmpC Bla. The probe can clearly distinguish both the susceptibility of the tested strains by inhibition zone and resistant mechanism by the fluorescence response to different bacterial strains in solution. Therefore, **CDC-559** provides the means to screen AmpC Bla inhibitors rapidly and to determine the susceptibility of bacterial strains against β-lactams and possible resistance mechanisms, which is one of the requirements of precision medicine.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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